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REMARKS

Claims 27-40 are pending in the subject application. By this Amendment, applicants have amended claims 27, 28, 33 and 39 to recite the descriptor "human" in relation to Ku70. Support for this can be found in the specification as originally filed at, inter alia, page 52, lines 18-33. In addition, applicants have amended claims 27, 28, 33 and 39 to recite that the antisense oligonucleotide has the sequence of a human Ku70 cDNA in the antisense orientation. Support for this can be found in the specification as originally filed at, inter alia, page 83, lines 7 to 16 and Fig. 13. In addition, applicants have amended claims 30, 34-36 and 38 merely to correct formatting errors.

Applicants maintain that the amendments to the claims raise no issue of new matter. After entry of this Amendment, claims 27-40 will still be pending and under examination.

Provisional Obviousness-Type Double Patenting Rejection

In the April 13, 2006 Office Action, the Examiner provisionally rejected claims 27, 39 and 40 under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1, 15, 16 and 18-22 of copending U.S. Application No. 09/750,410.

Applicants understand that this is only a provisional rejection, and will respond should the rejection become non-provisional.

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Rejections Under 35 U.S.C. §103(a)

The Examiner rejected claims 27, 39 and 40 as allegedly obvious over Reeves et al. (J. Biol. Chem., Vol. 26499):5047-5052, 1989) and Milner et al. (Nature Biotech. 15:537-541, 1997), the combination in view of Taniguchi et al. (actually Takiguchi et al.) (Genomics, 35:129-135, 1996) and AuYoung et al. (U.S. Patent No. 5,773,580) insofar as the claims are drawn to compositions and methods for increasing a target cell's sensitivity to DNA damaging agents in vitro comprising the administration of an antisense oligonucleotide, optionally in an adenoviral expression vector comprising a heat shock promoter, that specifically hybridizes with a nucleic acid encoding a DNA-dependent protein kinase subunit (Ku70) which antisense inhibits the expression of the target Ku70 subunit.

In response, applicants respectfully traverse the Examiner's rejection. Initially, applicants note that the rejected claims are not directed to methods or compositions involving a Ku70 antisense optionally in an adenoviral expression vector comprising a heat shock promoter as suggested by the Examiner. Instead, the adenoviral expression vector comprising a heat shock promoter is a recited element of all the rejected claims.

In order for an obviousness rejection of the claimed method under 35 U.S.C. 103(a) to be proper, the prior art references, in combination, must in part teach or suggest all the elements

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of the claimed invention. Applicants note, however, that the cited references in combination do not teach or suggest an adenoviral expression vector under control of a heat shock promoter comprising an antisense oligonucleotide that specifically hybridizes to a nucleic acid encoding Ku70 so as to prevent expression thereof, wherein the antisense has the sequence of a human Ku70 cDNA in the antisense orientation, as set forth in amended claims 40 and 39, and as employed in the method recited in amended claim 27.

With regard to the Examiner's statement that AuYong et al. teaches pharmaceutical compositions comprising antisense oligonucleotides as well as teaching adenoviral expression vectors comprising antisense oligonucleotides, applicants note that AuYoung et al. does not teach such, but instead discloses expression vectors comprising IPKC coding sequences. AuYoung et al. teaches methods of promoting expression of a protein using an expression vector (see col. 10-11, as cited by the Examiner, section entitled "*Expression Systems*"), rather than a method of inhibiting expression of a protein using an adenoviral expression vector. Furthermore, at col. 20-21 cited by the Examiner, AuYoung et al. discusses delivery of IPKC antisense into cells, but not in an adenoviral expression system under the control of a heat shock promoter. The remaining cited references in combination with AuYoung et al. do not make up for these deficiencies.

In short, the cited references in combination do not teach or

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suggest all of the elements of the claimed invention.

Rejection Under 35 U.S.C. §112, First Paragraph (Written Description)

The Examiner rejected claims 27-40 under 35 U.S.C. §112, first paragraph, as allegedly not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner indicated that the claims are drawn to compositions and methods comprising the administration of antisense oligonucleotides that specifically hybridize with any nucleic acid molecule encoding Ku70, inhibit its expression in vitro or in vivo, and provide for treatment effects in a subject, but that the claims allegedly do not adequately describe the distinguishing features or attributes concisely shared by the members of the genus.

In response, applicants respectfully traverse the Examiner's rejection. Applicants note that the compositions and method claims as amended encompass antisense oligonucleotides, or use thereof, that specifically hybridize to a nucleic acid encoding a human Ku70 so as to prevent expression thereof. As such, the members of the genus need to possess all of the structural features determined from being (i) an antisense oligonucleotide (ii) that specifically hybridizes to a specific human nucleic acid, (iii) that prevents expression thereof and wherein (iv) the antisense oligonucleotide has the sequence of a human Ku70

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cDNA in the antisense orientation. Thus, the members of the genus do not vary in the requisite structural features set forth in the claims and described in the specification. Furthermore, the human Ku70 gene sequence is known in the art. See Reeves et al. (1989), (**Exhibit 1**) and Genbank 51093847, (**Exhibit 2**).

Applicants maintain that those of skill in the art of the *claimed invention* would recognize from the description that the claimed antisense is described in the specification.

Thus, applicants maintain that the specification shows applicants were in possession of the claimed invention at the time of filing. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Rejection Under 35 U.S.C. §112, First Paragraph (Enablement)

The Examiner rejected claims 27-38 and 40 under 35 U.S.C. §112, first paragraph, as allegedly not enabled by the specification. The Examiner stated that the specification, while being enabling for an in vitro method of increasing a target cell's susceptibility to DNA damaging agents, does not reasonably provide for in vivo methods.

In response, applicants respectfully traverse the Examiner's rejection. Applicants note, with regard to amended claim 27, that a working example of the antisense increasing the

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susceptibility of a cell to DNA-damaging agents is set forth at page 83, lines 7 to 16; and in Fig. 13 and the description thereof in the specification at page 12, lines 5 to 9.

Applicants further note that the references cited by the Examiner as evidence of the state of the art regarding antisense delivery in vivo do not address adenoviral mediated delivery, as recited in the claimed methods. Moreover, adenoviral mediated delivery of nucleic acids is recognized as effective (for example see U.S. Patent No. 5,773,580, as cited by the Examiner). In addition, applicants note that Crooke, cited by the Examiner, discusses a number of antisenses effective in vivo. See Table 1, and see accompanying text, pages 22-26.

Applicants maintain that the claims as amended are enabled by the specification and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Conclusion

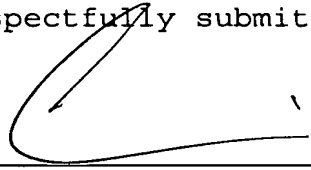
For the reasons set forth above, applicants respectfully request that the Examiner reconsider and withdraw the rejections, and solicit allowance of pending claims 27-40.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

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No fee, other than the \$510.00 extension fee, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:
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EXHIBIT 1

Molecular Cloning of cDNA Encoding the p70 (Ku) Lupus Autoantigen*

(Received for publication, October 5, 1988)

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The Ku (p70/p80) autoantigen consists of two phosphoproteins of molecular mass ~70,000 and 80,000 forming a macromolecular complex that binds DNA. Autoantibodies from a patient with systemic lupus erythematosus were used to isolate cDNA clones encoding the human ~70-kDa Ku antigen (p70) from a λ gt11 expression library. The deduced amino acid sequence of p70 consisted of 609 amino acid residues and was confirmed by partial amino acid sequencing. The protein contains two acidic domains of 61 residues (31% Glu + Asp) and 19 residues (53% Glu + Asp) that are similar in size and charge to those found in a number of proteins involved in transcriptional activation. The 61-residue acidic region is rich in serine, raising the possibility that its charge might be modulated by phosphorylation. The predicted amino acid sequence also contains two regions with periodic repeats of either leucine alone, or leucine alternating with serine every seventh position. The latter repeat displays sequence and secondary structural similarities with the "leucine zipper" regions of the *c-myc* and *v-myc* oncogene products. The p70 antigen does not appear to have extensive sequence homology with the 80-kDa Ku autoantigen based on analysis of RNA blots and immunological criteria. A major antigenic determinant or determinants recognized by human autoantibodies is located near a leucine repeat on the carboxyl-terminal 190 amino acid residues of p70.

The p70/p80 autoantigen consists of two proteins of molecular mass ~70,000 and ~80,000 daltons that dimerize to form a 10 S DNA-binding complex (1). Exchange of immunological reagents has established that the p70/p80 antigen (1, 2), Ku antigen (3-5), Ki antigen (6), as well as a 86-70-kDa protein complex (7, 8)¹ are identical. The p70/p80 complex binds to the ends of double-stranded DNA (4) in a cell cycle-dependent manner, being associated with chromosomes of interphase cells, followed by complete dissociation from the condensing

chromosomes in early prophase (2). Both p70 and p80 have been found to contain phosphoserine residues (8). The function of the antigen is unknown, but a role in DNA repair or transposition has been proposed (4, 5). Certain individuals with systemic lupus erythematosus (SLE)² and related disorders produce extremely large amounts of autoantibodies to p70 and p80 (1, 3, 6). We have used autoantibodies from the serum of an individual with SLE to isolate cDNA clones encoding p70, the protein that is thought to mediate binding of the Ku (p70/p80) complex to DNA (5). Analysis of the predicted amino acid sequence of p70 suggests structural similarities with other DNA-binding proteins. The amino acid sequence should be useful for examining the function of the Ku (p70/p80) complex, as well as the causes of autoimmunity to this antigen.

MATERIALS AND METHODS

Isolation of cDNA Clones—Human autoantibodies to the Ku (p70/p80) antigen from a patient (CK) with SLE were used to screen a human hepatoma λ gt11 cDNA library, provided by M. Mueckler (Whitehead Institute, Cambridge, MA), using established protocols (9-11). Recombinant phage were plated on lawns of *Escherichia coli* Y1090 and overlaid with nitrocellulose filters (Schleicher & Schuell, BA85) impregnated with isopropylthiogalactoside (Sigma). Positive plaques were detected by incubating in blocking solution (150 mM NaCl, 50 mM Tris, pH 7.5, 1% bovine hemoglobin, 0.02% NaN₃) for 1 h at 22 °C, followed by CK serum (1:5000 in blocking solution, which was preadsorbed with bacterial lysate) (11) for 8 h at 4 °C, and ¹²⁵I-protein A (Du Pont-New England Nuclear; 10⁵ dpm/ml) for 3 h at 22 °C. Three cDNA clones were obtained, the longest of which (~2.0 kb) was used to screen the same library by nucleic acid hybridization (12). Probes were labeled with [α -³²P]dCTP by random priming (13) using Klenow fragment (Amersham Corp.). In addition, a 27-bp oligonucleotide 5'-CTTCCTCTGCTTCTTCATCGCCCTCGG-3' complementary to the 5' end of the 2.0-kb clone was synthesized (Applied Biosystems 380A DNA synthesizer), ³²P end-labeled with polynucleotide kinase (14) and used to rescreen the library (15).

Production of p70 Fusion Proteins— λ gt11 clones 70.5, 70.34, and 70.77 were used to lysogenize *E. coli* Y1089, and fusion proteins were isolated as described (11). *E. coli* lysates containing the fusion proteins were analyzed on 8% SDS-polyacrylamide gels, and stained with Coomassie Brilliant Blue R250 (16).

Immunoblotting of the fusion proteins was performed as described (17). Blots were incubated in blocking solution for >1 h, followed by CK serum (1:250 dilution), or by the same dilution of CK serum plus an irrelevant autoimmune serum (patient JK) at a dilution of 1:250 for 3 h at 22 °C. After washing three times for 30 min, the blots were incubated with alkaline phosphatase-conjugated goat anti-human IgG antibodies (1:1500 dilution, from Tago, Burlingame, CA) for 3 h at 22 °C. Antibodies specific for the fusion proteins were purified by elution from the nitrocellulose blots (18) and used to probe immunoblots of K562 nuclear extract (2) followed by detection with ¹²⁵I-protein A as described above.

DNA Sequence Analysis—Restriction fragments of the phage cDNA inserts were subcloned into pUC 19, subsequently into

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J04611.

† Recipient of an Arthritis Investigator Award from the Arthritis Foundation. To whom correspondence should be addressed: the Rockefeller University, 1230 York Ave., New York, NY 10021.

¹ M. Yaneva, personal communication.

² The abbreviations used are: SLE, systemic lupus erythematosus; kb, kilobase(s); bp, base pair(s); SDS, sodium dodecyl sulfate.

M13mp18 or M13mp19 (19), and sequenced from both strands by the dideoxy chain termination method (20). The rapid deletion subcloning technique of Dale *et al.* (21) was utilized to generate a sequential series of overlapping clones for sequencing. Oligonucleotides were synthesized and used without further purification (22) as primers for sequencing certain large fragments. Modified T7 DNA polymerase (Sequenase, United States Biochemical Corp., Cleveland, OH) using dITP in place of dGTP (23) was used for dideoxy sequencing of DNA regions not adequately resolved with Klenow fragment.

Computer Sequence Analysis—Sequences were assembled and analyzed by computer programs provided by the BIONET National Computer Resource for Molecular Biology. The translated amino acid sequence of p70 was compared to sequences in the National Biomedical Research Foundation Protein Identification Resource (PIR) using the algorithms of Lipman and Pearson (24, 25). Statistical significance of alignments was evaluated using the RDF program (24).

Protein Sequencing—Ku (p70/p80) antigen was purified from $\sim 3.5 \times 10^6$ K562 cells as described.³ Protein A-Sepharose beads were coated with monoclonal antibody 162 (1) at 4 °C for 8 h, washed three times with 150 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 0.5% Nonidet P-40, 1 mg/ml ovalbumin, 0.02% NaN₃, and added to an extract of K562 cells (in 150 mM NaCl, 50 mM Tris, pH 7.5, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride) for 3 h at 4 °C. The beads were washed three times with 150 mM NaCl, 50 mM Tris, pH 7.5, 2 mM EDTA, 0.25 M sucrose, 2.5% Triton X-100, 0.5% SDS, then three times with 150 mM NaCl, 50 mM Tris, pH 7.5, 2 mM EDTA, and heated to 100 °C for 3 min in SDS sample buffer (16) before resolving on 10% SDS-polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue R-250, and gel slices containing p70 were excised. The protein was electroeluted from the gel exactly as described by Hunkapiller *et al.* (27).

Electroeluted p70 was cleaved with chymotrypsin (Worthington) as follows: approximately 7 μ g of p70 in 60 μ l of 0.125 M Tris, pH 6.8, 0.5% SDS, 10% glycerol, 0.0001% bromophenol blue was heated to 100 °C for 3 min before adding chymotrypsin to a final concentration of 17 μ g/ml. The sample was incubated for 30 min at 37 °C; digestion was terminated by the addition of SDS to 2.5% and dithiothreitol to 0.1 M. The sample was then heated to 55 °C for 10 min and loaded onto a 12.5% SDS-polyacrylamide gel.

After electrophoresis, intact p70 and chymotryptic peptides were transferred to polyvinylidene difluoride membrane (Immobilon, Whatman, Clifton, NJ) (28). After visualization by Coomassie Blue staining, p70 and p70 peptides of ~ 29 , 22, and 16 kDa were excised from the blot and subjected to automated Edman degradation with the Applied Biosystems model 470A gas-phase sequencer. The phenylhydantoin amino acid derivatives were identified and quantitated using a Hewlett Packard 1084 HPLC system.

RNA Blot Analysis—K562 poly(A)⁺ RNA (29, 30) was separated on 0.8% agarose gels containing 2.2 M formaldehyde (14), transferred to nitrocellulose, and baked for 90 min at 80 °C (31). DNA probes were labeled by random priming (13) as described above. RNA blots were prehybridized for 6–12 h at 42 °C in 5 \times SSPE (1 \times SSPE = 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM EDTA), 10 \times Denhardt's solution (1 \times = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 50% formamide, 0.4 mg/ml denatured sonicated salmon sperm DNA, 0.1% SDS before hybridizing for 30 h in the same solution containing probe at 10⁶ dpm/ml at 42 °C. The blots were washed at 65 °C with 2 \times SSC (1 \times SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.4), 0.1% SDS (three times, 10 min each) followed by 0.3 \times SSC, 0.1% SDS (three times, 45 min each), and exposed to X-ray film (XAR-5, Kodak, Rochester, NY) with Lightening Plus intensifying screens (Du Pont-New England Nuclear).

RESULTS

Isolation of cDNA Clones Encoding p70 Epitopes—A λ gt11 expression library was screened with serum from a patient (CK) with high titer anti-Ku (p70/p80) antibodies. This serum contains anti-Ku (p70/p80) antibodies at a titer of approximately 1:3 $\times 10^6$, along with low levels (1:1000 titer or less) of anti-RNP and anti-Sm antibodies (32). At the 1:5000 dilution used for screening, the serum was essentially monospecific for p70. Screening the λ gt11 library with this serum

yielded three positive plaques, designated clones 70.5, 70.34, and 70.77, respectively (Fig. 1). After plaque purification, *Eco*RI digestion of purified phage DNA demonstrated insert DNA fragments of approximately 1600 and 350 bp (clone 70.5), 900 bp (clone 70.34), and 700 bp (clone 70.77). On Southern blots, insert DNA from clone 70.77 hybridized with insert DNA from clone 70.34, and with the ~ 1600 -bp fragment from clone 70.5 (not shown). DNA sequence analysis (see below) confirmed that the three clones contained fragments of the same gene.

Nucleic acid hybridization screening yielded additional λ gt11 clones hybridizing with both the clone 70.77 insert and with the ~ 350 -bp fragment of clone 70.5. Restriction mapping suggested that two of these clones, designated 70.30 and 70.45 (Fig. 1) contained additional DNA sequences not contained by clone 70.5. Screening with the 5'-oligonucleotide failed to yield clones with longer inserts.

E. coli lysogenic for λ gt11 clones 70.34 and 70.77 produced fusion proteins of ~ 145 and ~ 140 kDa, respectively, after induction with isopropylthiogalactoside (Fig. 2). *E. coli* lysogenic for clone 70.5 produced only trace quantities of fusion protein (not shown). Autoantibodies from CK serum were affinity purified on nitrocellulose-bound 70.34 or 70.77 fusion proteins and used to probe immunoblots of total nuclear proteins (Fig. 3). The affinity-purified anti-70.34 and anti-70.77 antibodies specifically bound to p70 on immunoblots of total nuclear proteins, while autoantibodies in the original CK serum bound to both p70 and p80 (Fig. 3A). Addition of JK autoimmune serum to CK serum resulted in binding to additional proteins on immunoblots (Fig. 3B, CK+JK). The contaminating JK autoantibodies were removed by affinity purification on 70.34 and 70.77 (Fig. 3B), demonstrating the specificity of binding to the fusion proteins.

DNA Sequence—The nucleotide sequence of cDNAs from clones 70.5, 70.34, 70.77, 70.30, and 70.45 was determined from both strands using the sequencing strategy shown in Fig. 1. The nucleotide sequence (Fig. 4) contains a single open reading frame of 1,827 bp (from nucleotide 34 to 1,860), coding for 609 amino acids. The predicted molecular mass of the encoded p70 protein is 69,851, in close agreement with the apparent molecular mass of 70,000 estimated by SDS-polyacrylamide gel electrophoresis (1). The open reading frame is preceded by a 5'-untranslated region of 33 bp, and followed by a 3'-untranslated region of 294 bp terminating with a

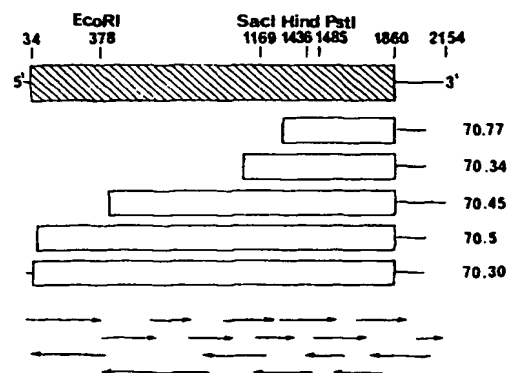


FIG. 1. p70 partial restriction map, clones, and sequencing strategy. The coding region (bases 34–1860) is shown as a hatched box in the partial restriction map (top). The individual cDNA clones obtained by screening with antibody probes are labeled 70.77 (bases 1286–2027), 70.34 (bases 1112–2025), and 70.5 (bases 44–2021). Additional cDNA clones obtained by nucleic acid hybridization are labeled 70.45 and 70.30. The sequencing strategy is indicated by arrows at the bottom.

³ W. H. Reeves, Z. M. Sthoeger, and R. G. Lahita, manuscript submitted for publication.

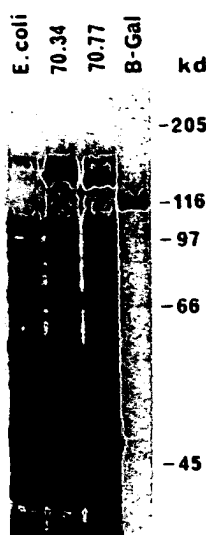


FIG. 2. SDS-polyacrylamide gel of fusion proteins obtained from *E. coli* Y1089 lysogenized by λ gt11 clones. *E. coli* were solubilized in SDS sample buffer, and proteins were resolved on an 8% SDS-polyacrylamide gel followed by Coomassie Blue staining. Lanes show *E. coli* Y1089 lysate, and lysates of *E. coli* Y1089 lysogenized by clones 70.34 and 70.77. The last lane shows purified β -galactosidase (Sigma) for comparison. Positions of molecular mass markers are indicated on the right. kD, kilodaltons.

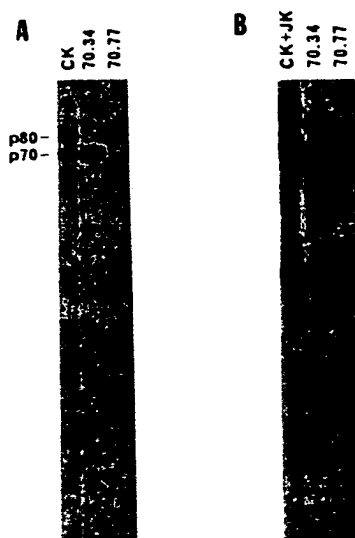


FIG. 3. Immunoblots of antibodies affinity-purified from blots of fusion proteins. A, immunoblots of K562 nuclear extract using CK serum (1:500) or CK antibodies (initial serum dilution 1:250) affinity-purified from 70.34 or 70.77 fusion proteins, respectively. On immunoblots of total nuclear extract, CK serum reacted with both p70 and p80, while the affinity-purified antibodies were specific for p70. B, immunoblots of K562 nuclear extract using CK plus JK sera (both at 1:500 dilution) or CK plus JK sera (initially each at 1:250) affinity-purified from 70.34 or 70.77 fusion proteins, respectively.

AATAAA sequence followed by a 68-bp poly(A) sequence. Two clones (70.5 and 70.44) had a cytidine at position 300, while two others (70.30 and 70.26) had a thymidine. The substitution does not change the predicted amino acid sequence and may represent allelic variation.

The sequence AACATG (nucleotides 31–36) is a potential ribosome binding site (33) which may encode the initiator methionine as indicated in Fig. 4. However, this prediction

could not be confirmed by amino acid sequencing because the amino terminus of p70 was blocked.

Partial Amino Acid Sequence of p70—Since the amino-terminal sequence of p70 was unobtainable, the protein was cleaved with chymotrypsin and partial amino acid sequences of peptides of molecular mass ~29, 27, and 16 kDa were determined. The amino acid sequences of the three peptides match the predicted amino acid sequence as shown in Fig. 4 (single letter code), confirming the identity of the cDNA clone.

RNA Blot Analysis—Probes consisting of the 3' ~1640 bp and 5' ~340 bp of clone 70.5 each hybridized with a single mRNA species of ~2.4 kb (Fig. 5, probes A and B, respectively). Thus, although the entire coding sequence has probably been determined, the sequence of the 5'-untranslated region is likely incomplete.

p70 Has a Cluster of Acidic Amino Acids and Periodic Repeats of Leucine or Leucine and Serine Residues—Examination of the predicted amino acid sequence of p70 revealed the existence of a high concentration of negatively charged residues near the amino terminus. The first 61 amino acids consist of 31% glutamic acid + aspartic acid, with a 19-amino acid region (residues 10–28, underlined in Fig. 4) consisting of 58% Glu + Asp. In addition, the amino-terminal 81 amino acids contains 13 serine residues (16%). A shorter acidic domain is present from residues 328–340 (7/13 residues or 53% Glu + Asp, underlined in Fig. 4).

Comparison of the amino acid sequence with known sequences in the National Biomedical Research Foundation Protein Identification Resource database revealed a possibly significant similarity with the v-myc oncogene product (Fig. 6). A region of p70 from amino acid 187 to 248 (62 residues) was 27% identical with a region of the v-myc oncogene protein from amino acid 361 to 422, and displayed weaker similarity with the c-myc protein. Statistical analysis of this alignment using the RDF program (24) gave an initial score of 62 ($z = 9.59$ S.D.) the aligned score of 62 ($z = 5.62$ S.D.). This region of both v-myc and c-myc contains a "leucine zipper" domain characterized by the periodic repetition of leucine residues every seventh position in an α -helical region (34). The p70 sequence has identical periodicity, but instead of having leucine residues at every seventh position, has leucine alternating with serine (Figs. 4, 6, and 7, indicated by *). Secondary structure predictions for p70, v-myc, and c-myc in this region are suggestive of α -helix formation (Fig. 7). Immediately adjacent to this region (toward the carboxyl terminus) is a 22-amino acid region containing 50% basic residues (Fig. 7, indicated by x), as appears in other proteins with leucine repeats (34). Another possible leucine repeat in p70 occurs from amino acids 483 to 511 (Fig. 4, residues at seventh positions indicated by *), but contains a proline residue (residue 500) that might destabilize a region of α -helix.

DISCUSSION

The Ku (p70/p80) antigen is recognized by autoantibodies in sera of certain patients with SLE (1) and other (3) collagen vascular diseases. The function of this antigen is not known, but previous studies have shown that the p70 and p80 proteins form a complex (1, 6, 7) that binds to DNA (1, 4, 5, 7). Binding to DNA may be mediated by p70 (5) and also be specific for ends of double-stranded DNA, suggesting a possible role in DNA repair or transposition (4).

These previous studies suggest that the p70 protein contains a region, or regions, mediating binding to DNA and to p80. As a first step to defining these regions, we have cloned and sequenced cDNA encoding p70. The translated amino acid sequence consists of 609 amino acids (Fig. 4). However, the

FIG. 4. Nucleotide and translated amino acid sequence of p70. DNA sequence is shown *above*, and predicted amino acid sequence *below* in *three-letter code*. Numbering corresponds to the predicted amino acid sequence. Amino acid sequences determined by automated Edmann degradation are indicated by *one-letter code* beneath the predicted amino acid sequence. Anionic domains of the translated protein (residues 11-29 and 330-342) are *underlined*. Periodic repeats of leucine and/or serine residues are indicated by *. A potential polyadenylation signal (AATAAA) is indicated (.....).

predicted initial methionine may be cleaved *in vivo*, since it is followed by serine, a residue that promotes removal of amino-terminal methionine residues by an amino-terminal methionine aminopeptidase (35). In addition, the amino terminus of p70 appears to be blocked. Acetylated methionine residues are generally not followed by serine (35, 36), while an amino-terminal serine residue is frequently acetylated (37), providing further indirect evidence that the amino-terminal residue *in vivo* may be serine rather than methionine.

Analysis of the predicted p70 amino acid sequence demonstrated two regions of possible α -helical secondary structure (Fig. 7) containing periodic repeats of either leucine and serine (residues 215–243) or leucine alone (residues 483–504) (Figs. 4 and 6). The Leu-Ser repeat region of p70 displays a possibly significant sequence similarity with a region of the v-myc and c-myc proteins that is essential for transformation (38), and which contains a leucine repeat with identical periodicity.

While the functional significance of this similarity is difficult to assess at present, it is notable that two cellular differentiation factors, the MyoD1 protein (39) and the T4 achaete-scute protein of *Drosophila* (40), also display comparable similarities with this region of *myc*.

The Leu- and Leu-Ser repeat regions of p70 are similar to leucine repeat regions found in a number of oncogene products and transcription factors (34). Many of these proteins contain a region rich in basic amino acids immediately adjacent to the leucine repeat. The Leu-Ser repeat of p70 is adjacent to a strongly basic region (Fig. 7) and the leucine repeat to a less strongly basic region (residues 461-482). In the model proposed by Landschulz *et al.* (34), the periodic repeat of leucine residues is thought to interdigitate with a similar domain of a second protein, juxtaposing the basic amino acids of the two proteins in a manner suitable for sequence-specific recognition of DNA. It remains to be determined whether either the

[illegible]

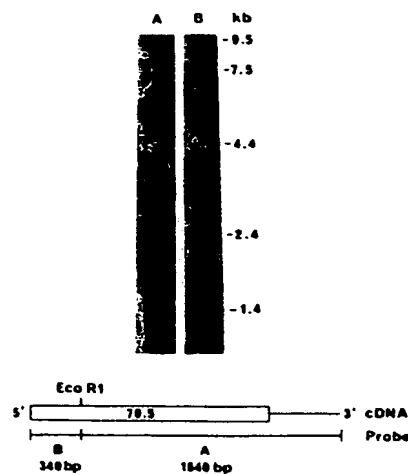


FIG. 5. RNA blots of K562 poly(A)⁺. Poly(A)⁺ RNA (13.2 µg/lane) was analyzed on a 1% agarose/formaldehyde gel and transferred to nitrocellulose. Blots were baked, prehybridized, and hybridized with ³²P-labeled EcoRI fragments of clone 70.5: A = ~1640 bp 3' fragment; B = ~340 bp 5' fragment. Both fragments hybridized with a RNA species of ~2.4 kb. Positions of RNA standards (Bethesda Research Laboratories, Gaithersburg, MD) are indicated.

p70 RTKAGDLRDTGIFLDLMLKKPGGDISLFYRDIISIAEDEDLRVHFESSKLEDLLRKVRA
v-myc RDQIPEVANNEKAPKVVILKKATEYVLSQSDENKLI AEKEQLRRRREQLKHNLEQLRNSRA
c-myc RDQIPELENNEKAPKVVILKKATAYILSVQAEQKLI SEEDLLRRRREQLKHNLEQLRNSCA

FIG. 6. Amino acid sequence similarity between p70, v-myc, and c-myc. The deduced amino acid sequence of p70 (residues 187–248) was aligned to maximize similarity with the amino acid sequences of v-myc (avian myelocytomatosis virus) (49), residues 361–422, and human c-myc (50), residues 399–460. This region of similarity coincides with the proposed "leucine zipper" domain of the myc proteins (34). Positions of the periodic repeats of leucine and serine (p70) or leucine alone (v-myc and c-myc) are indicated by *.

p70
RTKAGDLRDTGIFLDLMLKKPGGDISLFYRDIISIAEDEDLRVHFESSKLEDLLRKVRAKETRKAALSRLKLNK
A AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA
TTTT TTTT BBBBBB BBBBBB BBBBBB BBBBBB BBBBBB BBBBBB BBBBBB BBBBBB BBBBBB
v-myc (Avian Myelocytomatosis Virus)
RDQIPEVANNEKAPKVVILKKATEYVLSQSDENKLI AEKEQLRRRREQLKHNLEQLRNSRA
AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA
BBBBBB BBBBBB TTTT TTTT
c-myc (Human)
RDQIPELENNEKAPKVVILKKATAYILSVQAEQKLI SEEDLLRRRREQLKHNLEQLRNSCA
AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA
BBBBBB BBBBBB TTTT

FIG. 7. Predicted secondary structures of similar regions of p70, v-myc, and c-myc. A denotes helix-permissive structure, B denotes β-sheet, and T denotes turn, as predicted by the program of Chou and Fasman (26). Positions of periodic repeats of leucine and serine (p70) or leucine alone (v-myc and c-myc) are indicated (*). Basic residues in a 22-amino acid region immediately following the leucine-serine repeat of p70 are indicated by x.

leucine repeat or the Leu-Ser repeat can participate in the formation of this hypothetical structure. In particular, we cannot be certain that a polar amino acid such as serine would be compatible with the interdigitation postulated by the Land-schulz model. The sequence similarity of p70 with the leucine zipper region of myc, the α-helical secondary structure predicted for this region (Fig. 7), and the adjacent 22-residue basic domain may provide indirect evidence supporting this

possibility. Clearly, however, further experimental evidence will be necessary to assess the functional significance, if any, of this region. If either of these repeats is involved in the formation of a leucine zipper, then the Landschulz model would predict the existence of a similar region(s) in the p80 protein. This prediction will be readily testable when the sequence of p80 is available.

The predicted amino acid sequence of p70 also contains two regions rich in acidic residues (61 residues, 31% Glu + Asp, and 19 residues, 58% Glu + Asp, see Fig. 4). These acidic regions are comparable in length and charge to the acidic domains found in GCN4 (60 amino acids, 30% Glu + Asp) (41), and GAL4 (29 residues, 31% Glu + Asp, and 20 residues, 35% Glu + Asp) (42) that are thought to play a critical role in transcriptional activation (41–43). In addition, the high frequency of serine residues in the 61-amino acid acidic domain raises the possibility that the negative charge of this region might be increased by phosphorylation. Since the acidity of an "acid blob" appears to correlate with its transcriptional potency (44), phosphorylation of this region, if it occurs, might have functional significance. Thus, the structure of p70 resembles that of GCN4 and myc proteins not only in containing one or more possible leucine zipper domains (34, 41), but also in containing an anionic region (41, 45). Based on the existence of both a possible DNA-binding domain(s) and a potential transcriptional activator domain (43), it is tempting to speculate that p70 might have a role in transcription. Alternatively, the structure of p70 might be consistent with a role in DNA repair (4) or replication. These possibilities are not mutually exclusive, since recent studies indicate that certain transcriptional activators may be components of eukaryotic origins of DNA replication (46, 47).

The present studies demonstrate the existence of a major autoantigenic epitope or epitopes on the carboxyl-terminal 190 amino acids of p70 (Fig. 3, 70.77), a region containing the leucine repeat region of p70 (Fig. 4). We have previously found that autoantibodies in certain autoimmune sera inhibit the binding of p70/p80 to DNA, and conversely, that binding of DNA to p70/p80 partially inhibits autoantibody binding in some cases (2). Thus, at least one of the regions predicted to have a possible role in DNA binding may also be an important autoepitope. Recent studies from our laboratory suggest that the majority of autoantibodies to p70 in most sera from patients with SLE are reactive with this region.^{3,4}

The observation that antibodies eluted from the 70.34 fusion protein were specific for p70, and displayed no cross-reactivity with p80 suggests that the carboxyl-terminal 239 residues of p70 may not have extensive homology with p80, an interpretation that is also supported by the observation that p70 cDNA hybridized with a single poly(A)⁺ RNA (Fig. 5). It seems unlikely, therefore, that p70 and p80 are derived from a single gene by an alternative splicing mechanism. The possibility that p70 is derived from proteolytic cleavage of p80 is also highly unlikely. The immunologic cross-reactivity of p70 and p80 previously reported (6) may therefore reflect a relatively short region of p80 amino acid sequence similarity, possibly near the amino terminus of p70. We have been unable to test this possibility due to difficulties obtaining fusion proteins containing the amino-terminal 115 amino acids of p70. Although clone 70.5 contains these residues and was obtained by antibody screening, only trace amounts of fusion protein were produced by *E. coli* Y1089 lysogenized by this clone. Furthermore, we have been unable to express this region in a variety of plasmid expression vectors.⁴ The difficulty in expressing this region might relate to amino acid

⁴ W. H. Reeves and Z. M. Stoecker, unpublished observations.

sequences analogous to those that target certain proteins for rapid degradation in eukaryotic cells (48), or to low levels of synthesis and/or a high rate of degradation of the mRNA. Direct comparison of the sequence of p70 with that of p80, when available, may be necessary to localize the region(s) of immunologic similarity (6) between the two proteins. How autoimmunity to the p70 antigen develops, why it is closely linked to autoimmunity to p80, and whether the function of p70/p80 is related to the development of autoimmunity to the complex remain unanswered questions. The availability of the cloned autoantigens may be valuable in addressing these issues.


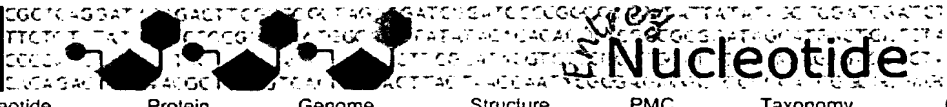
Acknowledgments—We are grateful to Dr. Günter Blobel for advice and critical reading of the manuscript. We are also indebted to Drs. Nilabh Chaudhary, Gregory Shelness, and Richard Wozniak for advice in DNA sequencing, Dr. Gary Greenberg for assistance in the use of computer programs, and Donna Atherton for determining the partial amino acid sequences of p70 proteolytic fragments and for the synthesis of oligonucleotides.

REFERENCES

- Reeves, W. H. (1985) *J. Exp. Med.* **161**, 18–39
- Reeves, W. H. (1987) *J. Rheumatol.* **14**, Suppl. 13, 97–105
- Mimori, T., Akizuki, M., Yamagata, H., Inada, S., Yoshida, S., and Homma, M. (1981) *J. Clin. Invest.* **68**, 611–620
- Mimori, T., and Hardin, J. A. (1986) *J. Biol. Chem.* **261**, 10375–10379
- Mimori, T., Hardin, J. A., and Steitz, J. A. (1986) *J. Biol. Chem.* **261**, 2274–2278
- Francoeur, A. M., Peebles, C. L., Gompper, P. T., and Tan, E. M. (1986) *J. Immunol.* **136**, 1648–1653
- Yaneva, M., Ochs, R., McRorie, D. K., Zweig, S., and Busch, H. (1985) *Biochim. Biophys. Acta* **841**, 22–29
- Yaneva, M., and Busch, H. (1986) *Biochemistry* **25**, 5057–5063
- Young, R. A., and Davis, R. W. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80**, 1194–1198
- Young, R. A., and Davis, R. W. (1983) *Science (Wash. D.C.)* **222**, 778–782
- Huynh, T. V., Young, R. A., and Davis, R. W. (1985) in *DNA Cloning: A Practical Approach* (Glover, D. M., ed) pp. 49–78, Vol. 1, IRL Press, Washington, D.C.
- Benton, W. D., and Davis, R. W. (1977) *Science (Wash. D.C.)* **196**, 180–182
- Feinberg, A. P., and Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13
- Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Wallace, R. B., Johnson, M. J., Hirose, T., Miyake, T., Kawashima, E. H., and Itakura, K. (1981) *Nucleic Acids Res.* **9**, 879–894
- Weber, K., and Osborn, M. (1975) in *The Proteins* (Neurath, H., and Hill, R. L., eds) 3rd Ed., pp. 179–223, Academic Press, New York
- Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 4350–4354
- Smith, D. E., and Fisher, P. E. (1984) *J. Cell Biol.* **99**, 20–28
- Norrander, J., Kempe, T., and Messing, J. (1983) *Gene (Amst.)* **26**, 101–106
- Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5463–5467
- Dale, R. M. K., McClure, B. A., and Houchins, J. P. (1985) *Plasmid* **13**, 31–40
- Urdea, M. S., and Sanchez-Pescador, R. (1987) *BioTechniques* **5**, 106–107
- Tabor, S., and Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 4767–4771
- Lipman, D. J., and Pearson, W. R. (1985) *Science (Wash. D.C.)* **227**, 1435–1441
- Pearson, W. R., and Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 2444–2448
- Chou, P. Y., and Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47**, 251–276
- Hunkapiller, M. W., Lujan, E., Ostrander, F., and Hood, L. E. (1983) *Methods Enzymol.* **93**, 227–236
- Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
- Aviv, H., and Leder, P. (1972) *Proc. Natl. Acad. Sci. U. S. A.* **69**, 1408–1412
- Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 5201–5205
- Reeves, W. H., and Chiorazzi, N. (1986) *J. Exp. Med.* **164**, 1029–1042
- Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872
- Landschulz, W. H., Johnson, P. F., and McKnight, S. L. (1988) *Science (Wash. D.C.)* **240**, 1759–1764
- Flinta, C., Persson, B., Jornvall, H., and von Heijne, G. (1986) *Eur. J. Biochem.* **154**, 193–196
- Tsunasawa, S., Stewart, J. W., and Sherman, F. (1985) *J. Biol. Chem.* **260**, 5382–5391
- Persson, B., Flinta, C., von Heijne, G., and Jornvall, H. (1985) *Eur. J. Biochem.* **152**, 523–527
- Stone, J., deLange, T., Ramsay, G., Jakobovits, E., Bishop, J. M., Varmus, H., and Lee, W. (1987) *Mol. Cell Biol.* **7**, 1697–1709
- Davis, R. L., Weintraub, H., and Lassar, A. B. (1987) *Cell* **51**, 987–1000
- Villares, R., and Cabrera, C. V. (1987) *Cell* **50**, 415–424
- Hope, I. A., and Struhl, K. (1986) *Cell* **46**, 885–894
- Ma, J., and Ptashne, M. (1987) *Cell* **48**, 847–853
- Sigler, P. B. (1988) *Nature* **333**, 210–212
- Triezenberg, S. J., Kingsbury, R. C., and McKnight, S. L. (1988) *Genes Dev.* **2**, 718–729
- Earnshaw, W. C. (1987) *J. Cell Biol.* **105**, 1479–1482
- DePamphilis, M. L. (1988) *Cell* **52**, 635–638
- O'Neill, E. A., Fletcher, C., Burrow, C. R., Heintz, N., Roeder, R. G., and Kelly, T. J. (1988) *Science (Wash. D.C.)* **241**, 1210–1213
- Rogers, S., Wells, R., and Rechsteiner, M. (1986) *Science (Wash. D.C.)* **234**, 364–368
- Alitalo, K., Bishop, J. M., Smith, D. H., Chen, E. Y., Colby, W. W., and Levinson, A. D. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80**, 100–104
- Watson, D. K., Psallidopoulos, M. C., Samuel, K. P., Dalla-Favera, R., and Papas, T. S. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80**, 3642–3645

Exhibit 2

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☐ 1: [NM_001469](#). Reports Homo sapiens X-ra...[gi:51093847]

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LOCUS NM_001469 2156 bp mRNA linear PRI 17-SEP-2006
 DEFINITION Homo sapiens X-ray repair complementing defective repair in Chinese hamster cells 6 (Ku autoantigen, 70kDa) (XRCC6), mRNA.
 ACCESSION NM_001469
 VERSION NM_001469.3 GI:51093847
 KEYWORDS .
 SOURCE Homo sapiens (human)
 ORGANISM [Homo sapiens](#)
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 2156)
 AUTHORS Silvera,D., Koloteva-Levine,N., Burma,S. and Elroy-Stein,O.
 TITLE Effect of Ku proteins on IRES-mediated translation
 JOURNAL Biol. Cell 98 (6), 353-361 (2006)
 PUBMED [16448389](#)
 REMARK GeneRIF: The present study suggests that Ku binds IRES -(internal ribosomal entry site)elements within RNA molecules, and that Ku plays a role in the modulation of IRES- mediated mRNA translation.
 REFERENCE 2 (bases 1 to 2156)
 AUTHORS Spagnolo,L., Rivera-Calzada,A., Pearl,L.H. and Llorca,O.
 TITLE Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair
 JOURNAL Mol. Cell 22 (4), 511-519 (2006)
 PUBMED [16713581](#)
 REMARK GeneRIF: Dimeric particles are observed in which two DNA-PKcs/Ku70/Ku80 holoenzymes interact through the N-terminal HEAT repeats.
 REFERENCE 3 (bases 1 to 2156)
 AUTHORS Zhu,P., Zhang,D., Chowdhury,D., Martinvalet,D., Keefe,D., Shi,L. and Lieberman,J.
 TITLE Granzyme A, which causes single-stranded DNA damage, targets the double-strand break repair protein Ku70
 JOURNAL EMBO Rep. 7 (4), 431-437 (2006)
 PUBMED [16440001](#)
 REMARK GeneRIF: Ku70 has other antiapoptotic functions in Granzyme A (GzmA)-induced cell death, which are blocked when GzmA proteolyses Ku70
 REFERENCE 4 (bases 1 to 2156)
 AUTHORS Martinez,J.J., Seveau,S., Veiga,E., Matsuyama,S. and Cossart,P.
 TITLE Ku70, a component of DNA-dependent protein kinase, is a mammalian receptor for Rickettsia conorii
 JOURNAL Cell 123 (6), 1013-1023 (2005)

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PUBMED 16360032
 REMARK GeneRIF: Ku70 is a receptor for the rickettsial protein rOmpB. Bacterial internalization is dependent on the presence of cholesterol-enriched microdomains containing Ku70.

REFERENCE 5 (bases 1 to 2156)
 AUTHORS Lee, J.C., Lee, C.H., Su, C.L., Huang, C.W., Liu, H.S., Lin, C.N. and Won, S.J.
 TITLE Justicidin A decreases the level of cytosolic Ku70 leading to apoptosis in human colorectal cancer cells
 JOURNAL Carcinogenesis 26 (10), 1716-1730 (2005)
 PUBMED 15905197
 REMARK GeneRIF: The level of Ku70 in the cytoplasm was decreased, but that of Bax in mitochondria was increased by justicidin A in colorectal cancer cells.

REFERENCE 6 (bases 1 to 2156)
 AUTHORS Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F.H., Goehler, H., Stroedicke, M., Zenkner, M., Schoenherr, A., Koeppen, S., Timm, J., Mintzlauff, S., Abraham, C., Bock, N., Kietzmann, S., Goedde, A., Toksoz, E., Droege, A., Krobitsch, S., Korn, B., Birchmeier, W., Lehrach, H. and Wanker, E.E.
 TITLE A human protein-protein interaction network: a resource for annotating the proteome
 JOURNAL Cell 122 (6), 957-968 (2005)
 PUBMED 16169070

REFERENCE 7 (bases 1 to 2156)
 AUTHORS Feki, A., Jefford, C.E., Berardi, P., Wu, J.Y., Cartier, L., Krause, K.H. and Irminger-Finger, I.
 TITLE BARD1 induces apoptosis by catalysing phosphorylation of p53 by DNA-damage response kinase
 JOURNAL Oncogene 24 (23), 3726-3736 (2005)
 PUBMED 15782130

REFERENCE 8 (bases 1 to 2156)
 AUTHORS Ting, N.S., Yu, Y., Pohorelic, B., Lees-Miller, S.P. and Beattie, T.L.
 TITLE Human Ku70/80 interacts directly with hTR, the RNA component of human telomerase
 JOURNAL (er) Nucleic Acids Res. 33 (7), 2090-2098 (2005)
 PUBMED 15824061
 REMARK GeneRIF: Ku70/80 interacts directly with the RNA component of human telomerase, independent of the human telomerase reverse transcriptase protein.

REFERENCE 9 (bases 1 to 2156)
 AUTHORS Ayene, I.S., Ford, L.P. and Koch, C.J.
 TITLE Ku protein targeting by Ku70 small interfering RNA enhances human cancer cell response to topoisomerase II inhibitor and gamma radiation
 JOURNAL Mol. Cancer Ther. 4 (4), 529-536 (2005)
 PUBMED 15827325
 REMARK GeneRIF: Ku70 has a role in human cancer cell sensitization to radiation and etoposide treatments

REFERENCE 10 (bases 1 to 2156)
 AUTHORS Mayeur, G.L., Kung, W.J., Martinez, A., Izumiya, C., Chen, D.J. and Kung, H.J.
 TITLE Ku is a novel transcriptional recycling coactivator of the androgen receptor in prostate cancer cells
 JOURNAL J. Biol. Chem. 280 (11), 10827-10833 (2005)
 PUBMED 15640154

REFERENCE 11 (bases 1 to 2156)
 AUTHORS Mischo, H.E., Hemmerich, P., Grosse, F. and Zhang, S.
 TITLE Actinomycin D induces histone gamma-H2AX foci and complex formation of gamma-H2AX with Ku70 and nuclear DNA helicase II

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JOURNAL J. Biol. Chem. 280 (10), 9586-9594 (2005)
 PUBMED 15613478
 REMARK GeneRIF: Histone gamma-H2AX promotes binding of nuclear DNA
 helicase II to transcriptionally stalled sites on chromosomal DNA.
 REFERENCE 12 (bases 1 to 2156)
 AUTHORS Wang,Q., Zhang,Z., Blackwell,K. and Carmichael,G.G.
 TITLE Vigilins bind to promiscuously A-to-I-edited RNAs and are involved
 in the formation of heterochromatin
 JOURNAL Curr. Biol. 15 (4), 384-391 (2005)
 PUBMED 15723802
 REFERENCE 13 (bases 1 to 2156)
 AUTHORS Andersen,J.S., Lam,Y.W., Leung,A.K., Ong,S.E., Lyon,C.E.,
 Lamond,A.I. and Mann,M.
 TITLE Nucleolar proteome dynamics
 JOURNAL Nature 433 (7021), 77-83 (2005)
 PUBMED 15635413
 REFERENCE 14 (bases 1 to 2156)
 AUTHORS Goehler,H., Lalowski,M., Stelzl,U., Waelter,S., Stroedicke,M.,
 Worm,U., Droege,A., Lindenberg,K.S., Knoblich,M., Haenig,C.,
 Herbst,M., Suopanki,J., Scherzinger,E., Abraham,C., Bauer,B.,
 Hasenbank,R., Fritzsche,A., Ludewig,A.H., Bussow,K., Coleman,S.H.,
 Gutekunst,C.A., Landwehrmeyer,B.G., Lehrach,H. and Wanker,E.E.
 TITLE A protein interaction network links GIT1, an enhancer of huntingtin
 aggregation, to Huntington's disease
 JOURNAL Mol. Cell 15 (6), 853-865 (2004)
 PUBMED 15383276
 REMARK Erratum: [Mol. Cell. 2005 Jul 22;19(2):287. Buessow, Konrad
 [corrected to Bussow, Konrad]]
 REFERENCE 15 (bases 1 to 2156)
 AUTHORS Beausoleil,S.A., Jedrychowski,M., Schwartz,D., Elias,J.E.,
 Villen,J., Li,J., Cohn,M.A., Cantley,L.C. and Gygi,S.P.
 TITLE Large-scale characterization of HeLa cell nuclear phosphoproteins
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 101 (33), 12130-12135 (2004)
 PUBMED 15302935
 REFERENCE 16 (bases 1 to 2156)
 AUTHORS Diederichs,S., Baumer,N., Ji,P., Metzelder,S.K., Idos,G.E.,
 Cauvet,T., Wang,W., Moller,M., Pierschalski,S., Gromoll,J.,
 Schrader,M.G., Koeffler,H.P., Berdel,W.E., Serve,H. and
 Muller-Tidow,C.
 TITLE Identification of interaction partners and substrates of the cyclin
 A1-CDK2 complex
 JOURNAL J. Biol. Chem. 279 (32), 33727-33741 (2004)
 PUBMED 15159402
 REFERENCE 17 (bases 1 to 2156)
 AUTHORS Sawchuk,D.J., Mansilla-Soto,J., Alarcon,C., Singha,N.C., Langen,H.,
 Bianchi,M.E., Lees-Miller,S.P., Nussenzweig,M.C. and Cortes,P.
 TITLE Ku70/Ku80 and DNA-dependent protein kinase catalytic subunit
 modulate RAG-mediated cleavage: implications for the enforcement of
 the 12/23 rule
 JOURNAL J. Biol. Chem. 279 (28), 29821-29831 (2004)
 PUBMED 15123719
 REMARK GeneRIF: Results show that Ku70/Ku80 and DNA-dependent protein
 kinase catalytic subunit (DNA-PKcs) modulate RAG-mediated cleavage
 during V(D)J recombination.
 REFERENCE 18 (bases 1 to 2156)
 AUTHORS Colland,F., Jacq,X., Trouplin,V., Mougin,C., Groizeleau,C.,
 Hamburger,A., Meil,A., Wojcik,J., Legrain,P. and Gauthier,J.M.
 TITLE Functional proteomics mapping of a human signaling pathway
 JOURNAL Genome Res. 14 (7), 1324-1332 (2004)
 PUBMED 15231748

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REFERENCE 19 (bases 1 to 2156)
AUTHORS Murata,L.B., Dodson,M.S. and Hall,J.D.
TITLE A human cellular protein activity (OF-1), which binds herpes simplex virus type 1 origin, contains the Ku70/Ku80 heterodimer
JOURNAL J. Virol. 78 (14), 7839-7842 (2004)
PUBMED [15220460](#)
REMARK GeneRIF: DNA-binding component of human OF-1 (which binds Herpes simplex virus type 1 origin of replication) contains Ku70 and Ku80 proteins

REFERENCE 20 (bases 1 to 2156)
AUTHORS Wang,H., Fang,R., Cho,J.Y., Libermann,T.A. and Oettgen,P.
TITLE Positive and negative modulation of the transcriptional activity of the ETS factor ESE-1 through interaction with p300, CREB-binding protein, and Ku 70/86
JOURNAL J. Biol. Chem. 279 (24), 25241-25250 (2004)
PUBMED [15075319](#)
REMARK GeneRIF: activity of ESE-1 is positively and negatively modulated by other interacting proteins including Ku70, Ku86, p300, and CBP.

REFERENCE 21 (bases 1 to 2156)
AUTHORS Li,B., Navarro,S., Kasahara,N. and Comai,L.
TITLE Identification and biochemical characterization of a Werner's syndrome protein complex with Ku70/80 and poly(ADP-ribose) polymerase-1
JOURNAL J. Biol. Chem. 279 (14), 13659-13667 (2004)
PUBMED [14734561](#)
REMARK GeneRIF: (ADP-ribosyl)ation of Ku70/80 reduces the ability of this factor to stimulate WRN exonuclease, suggesting that covalent modification of Ku70/80 by PARP-1 may play a role in the regulation of the exonucleolytic activity of WRN.

REFERENCE 22 (bases 1 to 2156)
AUTHORS Monferran,S., Muller,C., Mourey,L., Frit,P. and Salles,B.
TITLE The Membrane-associated form of the DNA repair protein Ku is involved in cell adhesion to fibronectin
JOURNAL J. Mol. Biol. 337 (3), 503-511 (2004)
PUBMED [15019772](#)
REMARK GeneRIF: cell-surface Ku functions as an adhesion receptor for fibronectin; both Ku70 and Ku80 present a structural relationship with integrin I (or A) domains and the A1 and A3 domains of von Willebrand factor, domains known to be involved in Fn binding

REFERENCE 23 (bases 1 to 2156)
AUTHORS Korabiowska,M., Bauer,H., Quentin,T., Stachura,J., Cordon-Cardo,C. and Brinck,U.
TITLE Application of new in situ hybridization probes for Ku70 and Ku80 in tissue microarrays of paraffin-embedded malignant melanomas: correlation with immunohistochemical analysis
JOURNAL Hum. Pathol. 35 (2), 210-216 (2004)
PUBMED [14991539](#)
REMARK GeneRIF: Expression of both genes was down-regulated as melanoma progressed. In situ hybridization demonstrated more Ku70- and Ku80-positive cells than immunohistochemical methods, but the correlation between the two methods was highly significant (P <0.01).

REFERENCE 24 (bases 1 to 2156)
AUTHORS Lim,J.W., Kim,H. and Kim,K.H.
TITLE The Ku antigen-recombination signal-binding protein Jkappa complex binds to the nuclear factor-kappaB p50 promoter and acts as a positive regulator of p50 expression in human gastric cancer cells
JOURNAL J. Biol. Chem. 279 (1), 231-237 (2004)
PUBMED [14570916](#)
REMARK GeneRIF: Ku antigen interacts with RBP-Jkappa and NF-kappaB p50 may

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- act as a positive regulator of p50 expression in gastric cancer AGS cells.
- REFERENCE 25 (bases 1 to 2156)
 AUTHORS Collins,J.E., Wright,C.L., Edwards,C.A., Davis,M.P., Grinham,J.A., Cole,C.G., Goward,M.E., Aguado,B., Mallya,M., Mokrab,Y., Huckle,E.J., Beare,D.M. and Dunham,I.
 TITLE A genome annotation-driven approach to cloning the human ORFeome
 JOURNAL Genome Biol. 5 (10), R84 (2004)
 PUBMED [15461802](#)
- REFERENCE 26 (bases 1 to 2156)
 AUTHORS Park,E.J., Chan,D.W., Park,J.H., Oettinger,M.A. and Kwon,J.
 TITLE DNA-PK is activated by nucleosomes and phosphorylates H2AX within the nucleosomes in an acetylation-dependent manner
 JOURNAL Nucleic Acids Res. 31 (23), 6819-6827 (2003)
 PUBMED [14627815](#)
 REMARK GeneRIF: DNA-PK can be activated by nucleosomes through the ability of Ku to bind to the ends of nucleosomal DNA, and that the activated DNA-PK is capable of phosphorylating H2AX within the nucleosomes
- REFERENCE 27 (bases 1 to 2156)
 AUTHORS Song,J.Y., Lim,J.W., Kim,H., Morio,T. and Kim,K.H.
 TITLE Oxidative stress induces nuclear loss of DNA repair proteins Ku70 and Ku80 and apoptosis in pancreatic acinar AR42J cells
 JOURNAL J. Biol. Chem. 278 (38), 36676-36687 (2003)
 PUBMED [12867423](#)
 REMARK GeneRIF: DNA repair proteins Ku70 and Ku80 expression is lost in cell nucleus after oxidative stress
- REFERENCE 28 (bases 1 to 2156)
 AUTHORS Godelock,D.M., Jiang,K., Pereira,E., Russell,B. and Sanchez,Y.
 TITLE Regulatory interactions between the checkpoint kinase Chk1 and the proteins of the DNA-dependent protein kinase complex
 JOURNAL J. Biol. Chem. 278 (32), 29940-29947 (2003)
 PUBMED [12756247](#)
- REFERENCE 29 (bases 1 to 2156)
 AUTHORS Schaffer,A., Kim,E.C., Wu,X., Zan,H., Testoni,L., Salamon,S., Cerutti,A. and Casali,P.
 TITLE Selective inhibition of class switching to IgG and IgE by recruitment of the HoxC4 and Oct-1 homeodomain proteins and Ku70/Ku86 to newly identified ATTT cis-elements
 JOURNAL J. Biol. Chem. 278 (25), 23141-23150 (2003)
 PUBMED [12672812](#)
- REFERENCE 30 (bases 1 to 2156)
 AUTHORS Ko,L. and Chin,W.W.
 TITLE Nuclear receptor coactivator thyroid hormone receptor-binding protein (TRBP) interacts with and stimulates its associated DNA-dependent protein kinase
 JOURNAL J. Biol. Chem. 278 (13), 11471-11479 (2003)
 PUBMED [12519782](#)
- REFERENCE 31 (bases 1 to 2156)
 AUTHORS Calsou,P., Delteil,C., Frit,P., Drouet,J. and Salles,B.
 TITLE Coordinated assembly of Ku and p460 subunits of the DNA-dependent protein kinase on DNA ends is necessary for XRCC4-ligase IV recruitment
 JOURNAL J. Mol. Biol. 326 (1), 93-103 (2003)
 PUBMED [12547193](#)
 REMARK GeneRIF: Coordinated assembly of Ku and p460 subunits of the DNA-dependent protein kinase on DNA ends is necessary for XRCC4-ligase IV recruitment
- REFERENCE 32 (bases 1 to 2156)
 AUTHORS Kurosawa,A., Shinohara,K., Watanabe,F., Shimizu-Saito,K.,

Koiwai,O., Yamamoto,K. and Teraoka,H.
TITLE Human neutrophils isolated from peripheral blood contain Ku protein but not DNA-dependent protein kinase
JOURNAL Int. J. Biochem. Cell Biol. 35 (1), 86-94 (2003)
PUBMED 12467650
REMARK GeneRIF: Transcripts of Ku70 and Ku86 genes were detected by RT-PCR and Ku protein was localized in the nucleus of neutrophils as a heterodimer

REFERENCE 33 (bases 1 to 2156)
AUTHORS Chai,W., Ford,L.P., Lenertz,L., Wright,W.E. and Shay,J.W.
TITLE Human Ku70/80 associates physically with telomerase through interaction with hTERT
JOURNAL J. Biol. Chem. 277 (49), 47242-47247 (2002)
PUBMED 12377759
REMARK GeneRIF: Ku associates with hTERT, and this interaction may function to regulate the access of telomerase to telomeric DNA ends

REFERENCE 34 (bases 1 to 2156)
AUTHORS Lim,J.W., Kim,H. and Kim,K.H.
TITLE Expression of Ku70 and Ku80 mediated by NF-kappa B and cyclooxygenase-2 is related to proliferation of human gastric cancer cells
JOURNAL J. Biol. Chem. 277 (48), 46093-46100 (2002)
PUBMED 12324457
REMARK GeneRIF: role of expression in NF-kappaB activation and COX-2 expression

REFERENCE 35 (bases 1 to 2156)
AUTHORS Madani,N., Millette,R., Platt,E.J., Marin,M., Kozak,S.L., Bloch,D.B. and Kabat,D.
TITLE Implication of the lymphocyte-specific nuclear body protein Sp140 in an innate response to human immunodeficiency virus type 1
JOURNAL J. Virol. 76 (21), 11133-11138 (2002)
PUBMED 12368356

REFERENCE 36 (bases 1 to 2156)
AUTHORS Ohta,S., Shiomi,Y., Sugimoto,K., Obuse,C. and Tsurimoto,T.
TITLE A proteomics approach to identify proliferating cell nuclear antigen (PCNA)-binding proteins in human cell lysates. Identification of the human CHL12/RFCs2-5 complex as a novel PCNA-binding protein
JOURNAL J. Biol. Chem. 277 (43), 40362-40367 (2002)
PUBMED 12171929

REFERENCE 37 (bases 1 to 2156)
AUTHORS Norwitz,E.R., Xu,S., Xu,J., Spiryda,L.B., Park,J.S., Jeong,K.H., McGee,E.A. and Kaiser,U.B.
TITLE Direct binding of AP-1 (Fos/Jun) proteins to a SMAD binding element facilitates both gonadotropin-releasing hormone (GnRH) - and activin-mediated transcriptional activation of the mouse GnRH receptor gene
JOURNAL J. Biol. Chem. 277 (40), 37469-37478 (2002)
PUBMED 12145309

REFERENCE 38 (bases 1 to 2156)
AUTHORS Willis,D.M., Loewy,A.P., Charlton-Kachigian,N., Shao,J.S., Ornitz,D.M. and Towler,D.A.
TITLE Regulation of osteocalcin gene expression by a novel Ku antigen transcription factor complex
JOURNAL J. Biol. Chem. 277 (40), 37280-37291 (2002)
PUBMED 12145306
REMARK GeneRIF: regulates osteocalcin gene expression

REFERENCE 39 (bases 1 to 2156)
AUTHORS Niwa,J., Ishigaki,S., Hishikawa,N., Yamamoto,M., Doyu,M., Murata,S., Tanaka,K., Taniguchi,N. and Sobue,G.

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TITLE Dorfin ubiquitylates mutant SOD1 and prevents mutant SOD1-mediated neurotoxicity
 JOURNAL J. Biol. Chem. 277 (39), 36793-36798 (2002)
 PUBMED 12145308
 REFERENCE 40 (bases 1 to 2156)
 AUTHORS Zipper,L.M. and Mulcahy,R.T.
 TITLE The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm
 JOURNAL J. Biol. Chem. 277 (39), 36544-36552 (2002)
 PUBMED 12145307
 REFERENCE 41 (bases 1 to 2156)
 AUTHORS Koike,M.
 TITLE Dimerization, translocation and localization of Ku70 and Ku80 proteins
 JOURNAL J. Radiat. Res. 43 (3), 223-236 (2002)
 PUBMED 12518983
 REMARK Review article
 GeneRIF: The mechanism that regulates for nuclear localization of Ku70 and Ku80 appears to play, at least in part, a key role in regulating the physiological function of Ku in vivo.
 REFERENCE 42 (bases 1 to 2156)
 AUTHORS Korabiowska,M., Tscherny,M., Stachura,J., Ruschenburg,I., Cordon-Cardo,C. and Brinck,U.
 TITLE Relationship between DNA mismatch repair genes expression, Ku-genes expression and ploidy-related parameters in the progression of pigmented lesions of the skin
 JOURNAL In Vivo 16 (5), 317-321 (2002)
 PUBMED 12494870
 REMARK GeneRIF: In naevus cell naevi, significant correlations were found between Ku70/80 gene expression and some ploidy-related parameters.
 REFERENCE 43 (bases 1 to 2156)
 AUTHORS Karmakar,P., Snowden,C.M., Ramsden,D.A. and Bohr,V.A.
 TITLE Ku heterodimer binds to both ends of the Werner protein and functional interaction occurs at the Werner N-terminus
 JOURNAL Nucleic Acids Res. 30 (16), 3583-3591 (2002)
 PUBMED 12177300
 REMARK GeneRIF: Ku heterodimer binds to both ends of the Werner protein and functional interaction occurs at the Werner N-terminus
 REFERENCE 44 (bases 1 to 2156)
 AUTHORS Ma,Y. and Lieber,M.R.
 TITLE Binding of inositol hexakisphosphate (IP6) to Ku but not to DNA-PKcs
 JOURNAL J. Biol. Chem. 277 (13), 10756-10759 (2002)
 PUBMED 11821378
 REMARK GeneRIF: binding with inositol hexakisphosphate
 REFERENCE 45 (bases 1 to 2156)
 AUTHORS Arosio,D., Cui,S., Ortega,C., Chovanec,M., Di Marco,S., Baldini,G., Falaschi,A. and Vindigni,A.
 TITLE Studies on the mode of Ku interaction with DNA
 JOURNAL J. Biol. Chem. 277 (12), 9741-9748 (2002)
 PUBMED 11796732
 REMARK GeneRIF: Studies on the mode of Ku interaction with DNA
 REFERENCE 46 (bases 1 to 2156)
 AUTHORS Andersen,J.S., Lyon,C.E., Fox,A.H., Leung,A.K., Lam,Y.W., Steen,H., Mann,M. and Lamond,A.I.
 TITLE Directed proteomic analysis of the human nucleolus
 JOURNAL Curr. Biol. 12 (1), 1-11 (2002)
 PUBMED 11790298
 REFERENCE 47 (bases 1 to 2156)
 AUTHORS Kelavkar,U., Wang,S. and Badr,K.

TITLE Divergence in intracellular signaling between interleukin-4 (IL-4) and IL-13 in human cells localizes to monomeric/dimeric expression of a transcription factor, the lupus autoantigen 70/80, induced by both cytokines

JOURNAL Adv. Exp. Med. Biol. 507, 483-489 (2002)

PUBMED [12664629](#)

REMARK GeneRIF: This autoantigen is induced by a divergence in intracellular signaling between IL-4 and IL-13.

REFERENCE 48 (bases 1 to 2156)

AUTHORS Kelavkar,U., Wang,S. and Badr,K.

TITLE KU 70/80 lupus autoantigen is the transcription factor induced by interleukins (IL)-13 and -4 leading to induction of 15-lipoxygenase (15-LO) in human cells

JOURNAL Adv. Exp. Med. Biol. 507, 469-481 (2002)

PUBMED [12664628](#)

REMARK GeneRIF: This antigen acts as the transcription factor induced by interleukins (IL)-13 and -4 leading to induction of 15-lipoxygenase (15-LO) in human cells.

REFERENCE 49 (bases 1 to 2156)

AUTHORS Park,S.J., Oh,E.J., Yoo,M.A. and Lee,S.H.

TITLE Involvement of DNA-dependent protein kinase in regulation of stress-induced JNK activation

JOURNAL DNA Cell Biol. 20 (10), 637-645 (2001)

PUBMED [11749722](#)

REFERENCE 50 (bases 1 to 2156)

AUTHORS Walker,J.R., Corpina,R.A. and Goldberg,J.

TITLE Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair

JOURNAL Nature 412 (6847), 607-614 (2001)

PUBMED [11493912](#)

REFERENCE 51 (bases 1 to 2156)

AUTHORS Li,L., Olvera,J.M., Yoder,K.E., Mitchell,R.S., Butler,S.L., Lieber,M., Martin,S.L. and Bushman,F.D.

TITLE Role of the non-homologous DNA end joining pathway in the early steps of retroviral infection

JOURNAL EMBO J. 20 (12), 3272-3281 (2001)

PUBMED [11406603](#)

REFERENCE 52 (bases 1 to 2156)

AUTHORS Schild-Poulter,C., Pope,L., Giffin,W., Kochan,J.C., Ngsee,J.K., Traykova-Andonova,M. and Hache,R.J.

TITLE The binding of Ku antigen to homeodomain proteins promotes their phosphorylation by DNA-dependent protein kinase

JOURNAL J. Biol. Chem. 276 (20), 16848-16856 (2001)

PUBMED [11279128](#)

REFERENCE 53 (bases 1 to 2156)

AUTHORS Song,K., Jung,Y., Jung,D. and Lee,I.

TITLE Human Ku70 interacts with heterochromatin protein 1alpha

JOURNAL J. Biol. Chem. 276 (11), 8321-8327 (2001)

PUBMED [11112778](#)

REFERENCE 54 (bases 1 to 2156)

AUTHORS Balajee,A.S. and Geard,C.R.

TITLE Chromatin-bound PCNA complex formation triggered by DNA damage occurs independent of the ATM gene product in human cells

JOURNAL Nucleic Acids Res. 29 (6), 1341-1351 (2001)

PUBMED [11239001](#)

REFERENCE 55 (bases 1 to 2156)

AUTHORS Romero,F., Multon,M.C., Ramos-Morales,F., Dominguez,A., Bernal,J.A., Pintor-Toro,J.A. and Tortolero,M.

TITLE Human securin, hPTTG, is associated with Ku heterodimer, the regulatory subunit of the DNA-dependent protein kinase

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JOURNAL Nucleic Acids Res. 29 (6), 1300-1307 (2001)
PUBMED 11238996
REFERENCE 56 (bases 1 to 2156)
AUTHORS Pucci,S., Mazzarelli,P., Rabitti,C., Gai,M., Gallucci,M.,
Flammia,G., Alcini,A., Altomare,V. and Fazio,V.M.
TITLE Tumor specific modulation of KU70/80 DNA binding activity in breast
and bladder human tumor biopsies
JOURNAL Oncogene 20 (6), 739-747 (2001)
PUBMED 11314007
REFERENCE 57 (bases 1 to 2156)
AUTHORS Daniel,R., Katz,R.A., Merkel,G., Hittle,J.C., Yen,T.J. and
Skalka,A.M.
TITLE Wortmannin potentiates integrase-mediated killing of lymphocytes
and reduces the efficiency of stable transduction by retroviruses
JOURNAL Mol. Cell. Biol. 21 (4), 1164-1172 (2001)
PUBMED 11158303
REMARK Erratum: [Mol Cell Biol 2001 Apr;21(7):2617]
REFERENCE 58 (bases 1 to 2156)
AUTHORS Tang,D., Xie,Y., Zhao,M., Stevenson,M.A. and Calderwood,S.K.
TITLE Repression of the HSP70B promoter by NFIL6, Ku70, and MAPK involves
three complementary mechanisms
JOURNAL Biochem. Biophys. Res. Commun. 280 (1), 280-285 (2001)
PUBMED 11162511
REFERENCE 59 (bases 1 to 2156)
AUTHORS Baekelandt,V., Claeys,A., Cherepanov,P., De Clercq,E., De
Strooper,B., Nuttin,B. and Debyser,Z.
TITLE DNA-Dependent protein kinase is not required for efficient
lentivirus integration
JOURNAL J. Virol. 74 (23), 11278-11285 (2000)
PUBMED 11070027
REFERENCE 60 (bases 1 to 2156)
AUTHORS Song,K., Jung,D., Jung,Y., Lee,S.G. and Lee,I.
TITLE Interaction of human Ku70 with TRF2
JOURNAL FEBS Lett. 481 (1), 81-85 (2000)
PUBMED 10984620
REFERENCE 61 (bases 1 to 2156)
AUTHORS Nick McElhinny,S.A., Snowden,C.M., McCarville,J. and Ramsden,D.A.
TITLE Ku recruits the XRCC4-ligase IV complex to DNA ends
JOURNAL Mol. Cell. Biol. 20 (9), 2996-3003 (2000)
PUBMED 10757784
REFERENCE 62 (bases 1 to 2156)
AUTHORS Cooper,M.P., Machwe,A., Orren,D.K., Brosh,R.M., Ramsden,D. and
Bohr,V.A.
TITLE Ku complex interacts with and stimulates the Werner protein
JOURNAL Genes Dev. 14 (8), 907-912 (2000)
PUBMED 10783163
REFERENCE 63 (bases 1 to 2156)
AUTHORS Sartorius,C.A., Takimoto,G.S., Richer,J.K., Tung,L. and
Horwitz,K.B.
TITLE Association of the Ku autoantigen/DNA-dependent protein kinase
holoenzyme and poly(ADP-ribose) polymerase with the DNA binding
domain of progesterone receptors
JOURNAL J. Mol. Endocrinol. 24 (2), 165-182 (2000)
PUBMED 10750018
REFERENCE 64 (bases 1 to 2156)
AUTHORS Mahajan,K.N., Gangi-Peterson,L., Sorscher,D.H., Wang,J.,
Gathy,K.N., Mahajan,N.P., Reeves,W.H. and Mitchell,B.S.
TITLE Association of terminal deoxynucleotidyl transferase with Ku
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 96 (24), 13926-13931 (1999)
PUBMED 10570175

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REFERENCE 65 (bases 1 to 2156)
AUTHORS Goedecke,W., Eijpe,M., Offenberg,H.H., van Aalderen,M. and Heyting,C.
TITLE Mrell and Ku70 interact in somatic cells, but are differentially expressed in early meiosis
JOURNAL Nat. Genet. 23 (2), 194-198 (1999)
PUBMED [10508516](#)

REFERENCE 66 (bases 1 to 2156)
AUTHORS Gell,D. and Jackson,S.P.
TITLE Mapping of protein-protein interactions within the DNA-dependent protein kinase complex
JOURNAL Nucleic Acids Res. 27 (17), 3494-3502 (1999)
PUBMED [10446239](#)

REFERENCE 67 (bases 1 to 2156)
AUTHORS Morio,T., Hanissian,S.H., Bacharier,L.B., Teraoka,H., Nonoyama,S., Seki,M., Kondo,J., Nakano,H., Lee,S.K., Geha,R.S. and Yata,J.
TITLE Ku in the cytoplasm associates with CD40 in human B cells and translocates into the nucleus following incubation with IL-4 and anti-CD40 mAb
JOURNAL Immunity 11 (3), 339-348 (1999)
PUBMED [10514012](#)

REFERENCE 68 (bases 1 to 2156)
AUTHORS Yang,C.R., Yeh,S., Leskov,K., Odegaard,E., Hsu,H.L., Chang,C., Kinsella,T.J., Chen,D.J. and Boothman,D.A.
TITLE Isolation of Ku70-binding proteins (KUBs)
JOURNAL Nucleic Acids Res. 27 (10), 2165-2174 (1999)
PUBMED [10219089](#)

REFERENCE 69 (bases 1 to 2156)
AUTHORS Singleton,B.K., Torres-Arzayus,M.I., Rottinghaus,S.T., Taccioli,G.E. and Jeggo,P.A.
TITLE The C terminus of Ku80 activates the DNA-dependent protein kinase catalytic subunit
JOURNAL Mol. Cell. Biol. 19 (5), 3267-3277 (1999)
PUBMED [10207052](#)

REFERENCE 70 (bases 1 to 2156)
AUTHORS Daniel,R., Katz,R.A. and Skalka,A.M.
TITLE A role for DNA-PK in retroviral DNA integration
JOURNAL Science 284 (5414), 644-647 (1999)
PUBMED [10213687](#)

REFERENCE 71 (bases 1 to 2156)
AUTHORS Grandvaux,N., Grizot,S., Vignais,P.V. and Dagher,M.C.
TITLE The Ku70 autoantigen interacts with p40phox in B lymphocytes
JOURNAL J. Cell. Sci. 112 (PT 4), 503-513 (1999)
PUBMED [9914162](#)

REFERENCE 72 (bases 1 to 2156)
AUTHORS Baumann,P. and West,S.C.
TITLE DNA end-joining catalyzed by human cell-free extracts
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 95 (24), 14066-14070 (1998)
PUBMED [9826654](#)

REFERENCE 73 (bases 1 to 2156)
AUTHORS Kumaravel,T.S., Bharathy,K., Kudoh,S., Tanaka,K. and Kamada,N.
TITLE Expression, localization and functional interactions of Ku70 subunit of DNA-PK in peripheral lymphocytes and Nalm-19 cells after irradiation
JOURNAL Int. J. Radiat. Biol. 74 (4), 481-489 (1998)
PUBMED [9798959](#)

REFERENCE 74 (bases 1 to 2156)
AUTHORS Barlev,N.A., Poltoratsky,V., Owen-Hughes,T., Ying,C., Liu,L., Workman,J.L. and Berger,S.L.
TITLE Repression of GCN5 histone acetyltransferase activity via

bromodomain-mediated binding and phosphorylation by the Ku-DNA-dependent protein kinase complex

JOURNAL Mol. Cell. Biol. 18 (3), 1349-1358 (1998)
PUBMED [9488450](#)
REFERENCE 75 (bases 1 to 2156)
AUTHORS Bandyopadhyay,D., Mandal,M., Adam,L., Mendelsohn,J. and Kumar,R.
TITLE Physical interaction between epidermal growth factor receptor and DNA-dependent protein kinase in mammalian cells

JOURNAL J. Biol. Chem. 273 (3), 1568-1573 (1998)
PUBMED [9430697](#)
REFERENCE 76 (bases 1 to 2156)
AUTHORS Jin,S., Kharbanda,S., Mayer,B., Kufe,D. and Weaver,D.T.
TITLE Binding of Ku and c-Abl at the kinase homology region of DNA-dependent protein kinase catalytic subunit

JOURNAL J. Biol. Chem. 272 (40), 24763-24766 (1997)
PUBMED [9312071](#)
REFERENCE 77 (bases 1 to 2156)
AUTHORS Gu,Y., Jin,S., Gao,Y., Weaver,D.T. and Alt,F.W.
TITLE Ku70-deficient embryonic stem cells have increased ionizing radiosensitivity, defective DNA end-binding activity, and inability to support V(D)J recombination

JOURNAL Proc. Natl. Acad. Sci. U.S.A. 94 (15), 8076-8081 (1997)
PUBMED [9223317](#)
REFERENCE 78 (bases 1 to 2156)
AUTHORS Smider,V. and Chu,G.
TITLE The end-joining reaction in V(D)J recombination

JOURNAL Semin. Immunol. 9 (3), 189-197 (1997)
PUBMED [9200330](#)
REMARK Review article
REFERENCE 79 (bases 1 to 2156)
AUTHORS Warriar,N., Page,N. and Govindan,M.V.
TITLE Expression of human glucocorticoid receptor gene and interaction of nuclear proteins with the transcriptional control element

JOURNAL J. Biol. Chem. 271 (31), 18662-18671 (1996)
PUBMED [8702520](#)
REFERENCE 80 (bases 1 to 2156)
AUTHORS Chung,U., Igarashi,T., Nishishita,T., Iwanari,H., Iwamatsu,A., Suwa,A., Mimori,T., Hata,K., Ebisu,S., Ogata,E., Fujita,T. and Okazaki,T.
TITLE The interaction between Ku antigen and REF1 protein mediates negative gene regulation by extracellular calcium

JOURNAL J. Biol. Chem. 271 (15), 8593-8598 (1996)
PUBMED [8621488](#)
REFERENCE 81 (bases 1 to 2156)
AUTHORS Romero,F., Dargemont,C., Pozo,F., Reeves,W.H., Camonis,J., Gisselbrecht,S. and Fischer,S.
TITLE p95vav associates with the nuclear protein Ku-70

JOURNAL Mol. Cell. Biol. 16 (1), 37-44 (1996)
PUBMED [8524317](#)
REFERENCE 82 (bases 1 to 2156)
AUTHORS Tuteja,N., Tuteja,R., Ochem,A., Taneja,P., Huang,N.W., Simoncsits,A., Susic,S., Rahman,K., Marusic,L., Chen,J. et al.
TITLE Human DNA helicase II: a novel DNA unwinding enzyme identified as the Ku autoantigen

JOURNAL EMBO J. 13 (20), 4991-5001 (1994)
PUBMED [7957065](#)
REFERENCE 83 (bases 1 to 2156)
AUTHORS Kaczmariski,W. and Khan,S.A.
TITLE Lupus autoantigen Ku protein binds HIV-1 TAR RNA in vitro

JOURNAL Biochem. Biophys. Res. Commun. 196 (2), 935-942 (1993)

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PUBMED [8240370](#)
REFERENCE 84 (bases 1 to 2156)
AUTHORS Higashiura,M., Shimizu,Y., Tanimoto,M., Morita,T. and Yagura,T.
TITLE Immunolocalization of Ku-proteins (p80/p70): localization of p70 to nucleoli and periphery of both interphase nuclei and metaphase chromosomes
JOURNAL Exp. Cell Res. 201 (2), 444-451 (1992)
PUBMED [1639139](#)
REFERENCE 85 (bases 1 to 2156)
AUTHORS Griffith,A.J., Craft,J., Evans,J., Mimori,T. and Hardin,J.A.
TITLE Nucleotide sequence and genomic structure analyses of the p70 subunit of the human Ku autoantigen: evidence for a family of genes encoding Ku (p70)-related polypeptides
JOURNAL Mol. Biol. Rep. 16 (2), 91-97 (1992)
PUBMED [1608402](#)
REFERENCE 86 (bases 1 to 2156)
AUTHORS Reeves,W.H. and Stthoeger,Z.M.
TITLE Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen
JOURNAL J. Biol. Chem. 264 (9), 5047-5052 (1989)
PUBMED [2466842](#)
REFERENCE 87 (bases 1 to 2156)
AUTHORS Chan,J.Y., Lerman,M.I., Prabhakar,B.S., Isozaki,O., Santisteban,P., Kuppers,R.C., Oates,E.L., Notkins,A.L. and Kohn,L.D.
TITLE Cloning and characterization of a cDNA that encodes a 70-kDa novel human thyroid autoantigen
JOURNAL J. Biol. Chem. 264 (7), 3651-3654 (1989)
PUBMED [2917966](#)
COMMENT REVIEWED [REFSEQ](#): This record has been curated by NCBI staff. The reference sequence was derived from [CD683757.1](#), [AK055786.1](#), [BC018259.2](#) and [BC012154.2](#).
On Aug 10, 2004 this sequence version replaced gi:[20070134](#).

Summary: The p70/p80 autoantigen is a nuclear complex consisting of two subunits with molecular masses of approximately 70 and 80 kDa. The complex functions as a single-stranded DNA-dependent ATP-dependent helicase. The complex may be involved in the repair of nonhomologous DNA ends such as that required for double-strand break repair, transposition, and V(D)J recombination. High levels of autoantibodies to p70 and p80 have been found in some patients with systemic lupus erythematosus.
COMPLETENESS: complete on the 3' end.

FEATURES Location/Qualifiers
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